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Climate change in forest ecosystems: A field experiment addressing the effects of raising temperature and reduced rainfall on early life cycle stages of oaks

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ABSTRACT

Higher temperatures and reduced rainfalls that are expected with the advance of climate change can impair the emergence and establishment of tree seedlings in forest ecosystems. These climatic changes can also decrease the availability of soil resources and reduce the performance of seedlings. We evaluated these effects in a temperate forest from Mexico with two native oak species (*Quercus crassifolia* and *Quercus eduardii*). As recently emerged oak seedlings are highly sensitive to changing environmental conditions, our field experiment was conducted across the season in which seedling emergence occurs (October–February). In the field, we used open-top chambers to increase temperature and rainout shelters to reduce rainfall, while controls were exposed to the current climate. Experimental plots of both treatments were established beneath the forest canopy because most oaks recruit in understory habitats. In these plots, we sowed acorns of both species in October 2015 and recorded seedling emergence and survival until February 2016, also monitoring temperature, precipitation and contents of water and nitrogen in the soil. On seedlings that survived until the end of the experiment we measured their growth, photosynthetic efficiency and foliar contents of water, carbon and nitrogen. Both the emergence and survival of *Q. crassifolia* seedlings were lower in climate change plots than in controls, but no differences were found for *Q. eduardii*. However, seedlings of both species had lower growth rates, photosynthetic efficiencies and contents of water, nitrogen and carbon in climate change simulation plots. These results indicate that climate change can impair tree seedling establishment in oak forest, also suggesting that their development will be constrained by reduced water and nitrogen availability.

1. Introduction

The prevalence of forest ecosystems mainly depends on the emergence and establishment of tree seedlings, but climate change can alter these processes (Clark et al., 2016). In temperate forests from North America, most global circulation models predict that the average air temperature will rise 5 °C in the course of this century, while annual precipitation levels in these ecosystems are projected to decrease up to 20% (IPCC, 2013). These ecosystems will then face warmer and drier conditions in the future, but field experiments assessing how tree seedlings will deal with these changes are still scarce.

Water availability is a key factor that regulates the emergence of tree seedlings in forests and climate change can strongly impair this process (Pérez-Ramos et al., 2013; Clark et al., 2016). This is particularly critical for tree species that produce recalcitrant seeds because higher temperatures and lower precipitation levels can enhance seed water loss and induce embryo mortality, hence reducing seedling emergence (Zavala-Chávez, 2008; Joët et al., 2013). Further, if seedlings emerge under these conditions, they can display a number of functional responses that would ultimately reduce their performance in climate change scenarios. Increased temperatures usually enhance foliar transpiration but, when plants are also subjected to water deficits, stomatal closure is induced to avoid excessive water loss and carbon fixation rates are reduced due to low gas exchange (Chaves et al., 2002;

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Haldimann and Feller, 2004; Rodriguez-Dominguez et al., 2016). Climate change can also affect light-dependent processes of photosynthesis in tree seedlings. This is because elevated thermal and/or water stress reduce the flow of electrons in the thylakoid membrane of chloroplasts and this decreases the conversion of light energy into chemical energy for fixing carbon (Maxwell and Johnson, 2000; Baker and Oxborough, 2004). Thus, the combined effects of warmer and drier conditions expected in forest ecosystems can alter water and carbon balances of tree seedlings and reduce their survival.

The availability of soil nitrogen is another important factor that can be altered by climate change. Nitrogen is the most important mineral nutrient that regulates plant growth (Poorter et al., 2012) and its natural inputs in forest ecosystems mainly depend on the activity of soil bacteria (Brookshire et al., 2011). These microorganisms are extremely sensitive to changes in soil temperature and moisture and, thus, the expected variations in climatic conditions can modify their efficiency for mineralizing nitrogen (Brookshire et al., 2011; Chen et al., 2011). However, the net effects of climate change on soil nitrogen are still controversial. This is because warming may stimulate the metabolic activity of nitrogen-fixing bacteria and increase soil nitrogen contents (Melillo et al., 2002; Brookshire et al., 2011; Butler et al., 2012), but reductions in soil moisture content due to reduced water inputs are expected to cause the opposite effects (Borken and Matzner, 2009; Chen et al., 2011). Instead, from the perspective of tree seedlings, the combined effects of increasing temperatures and reduced precipitation levels can jeopardize their ability to acquire nitrogen irrespective of whether its concentrations increase or decrease in the future. This is because these climatic conditions can induce strong water deficits and decrease the mobility of the mineral forms of nitrogen in the soil matrix, reducing the capability of plants to uptake this resource through the roots (Rennenberg et al., 2009; He and Dijkstra, 2014). In consequence, nitrogen contents and growth rates of tree seedlings in forest ecosystems are likely to decrease with the advance of climate change.

This study assesses how climate change could affect the emergence, survival and performance of recently emerged oak seedlings in a temperate forest of Mexico. For this, we performed a field experiment in which temperature and precipitation were manipulated during the period of the year in which the emergence of oak seedlings occurs, as these early life-cycle stages of oaks are extremely sensitive to changing environmental conditions (Zavala-Chávez and García-Moya, 1996; Badano et al., 2011, 2015). In these ecosystems, adult oaks produce acorns (nut-type, single-seeded fruits) in spring and release them in late summer, concurring with the second half of the rainy season (Zavala-Chávez and García-Moya, 1996; González-Salvatierra et al., 2013; Badano et al., 2015). As seeds of Mexican oaks are extremely recalcitrant (embryos die if seed water content drops below 25–30%) and they require elevated hydration levels to germinate (above 80% of seed water content), seedling emergence in the field occurs in short time after acorns are released from parental trees (Zavala-Chávez and García-Moya, 1996; Zavala-Chávez, 2008). The development of recently emerged oak seedlings mainly depends on the nutritional reserves accumulated in their cotyledons, but they are extremely susceptible to die because of thermal and water stress (Badano et al., 2009; González-Salvatierra et al., 2013). Thus, we hypothesized that warmer and drier conditions expected in climate change scenarios will reduce the emergence and survival of oak seedlings, also decreasing their overall performance. As climate change can reduce nitrogen availability for plants, we also hypothesized that the content of nitrogen in seedlings subjected to climate change conditions will be lower than those of seedling growing under the current climate.

2. Materials and methods

2.1. Study area

Our experiment was conducted at National Park "El Potosí", located in the westernmost section of Sierra Madre Oriental (Sierra de Álvarez), state of San Luis Potosí, Mexico (include the file "Pérez Ruiz et al. Experimental sites.kmz" here). Climate in this region is temperate (mean annual temperature 18 °C) and up to 80% of rainfalls occurs between June and November (annual precipitation 500–600 mm), but sparse precipitation events can happen until January (Vargas-Márquez, 1997). Soils are shallow, with maximum depths of 10–15 cm, and vegetation is composed by semi-deciduous oak forests. In these ecosystems, despite the shallowness of soils, tree roots penetrate the bedrock through small fissures, anchoring these organisms to the substrate (Zavala-Chávez and García-Sánchez, 1999). As seedling emergence mainly occurs in the upper section of the soil profile of the forest understory (Badano et al., 2015), we located the experimental site beneath the canopy of a well-preserved oak forest dominated by *Quercus crassifolia* Humb. & Bonpl., *Quercus eduardii* Trel., and *Quercus jonesii* Trel. (21°55′10″ N, 100°19′34″ W, 2123 m elevation).

To determine how much climate will change in the study site, we computed the difference between current and future values of air temperature and precipitation using the geodatabases of Fernández-Eguiarte et al. (2012, 2014). These geodatabases provide the values of these two variables with a spatial resolution of 1-km^2 per pixel, where current values are monthly averages that integrate data from 1902 to 2011 (Fernández-Eguiarte et al., 2012) and future values are estimated for each month of the year with the HadGEM2-ES climate change model (Fernández-Eguiarte et al., 2014). Future values of air temperature and precipitation are provided for the period 2021–2040 and consider the four climate change scenarios (RCP2.6, RCP4.5, RCP6.0 and RCP8.5) proposed in the latest report of IPCC (2013). The HadGEM2-ES model is commonly used make climate change predictions across continental Mexico because it has lower deviations than other models when past climatic conditions are estimated, and this reduces uncertainty about the future climate (Fernández-Eguiarte et al., 2014). Because we focused on the earlier life-cycle stages of oaks, differences between current and future values of these climatic conditions were computed for the period of the year in which seedling emergence occurs, comprised between October and February. These estimates indicated that, during the tree recruitment season, air temperature in the study site will increase about 1.3–1.7 °C and precipitation will decrease by 11–18% (Table 1).

2.2. Experimental design

The field experiment was carried out between 17 October 2015 and 27 February 2016, comprising the entire period in which oak seedlings emerge and their early establishment occurs. For this, we draw a quadrant of 50 × 50 m in the study site and randomly selected 20 point-sites within it on September 2015, taking care of maintaining a minimum distance of 5 m among them. Leaf litter and rocks were removed in a radius of 3 m around each of these sites. The half of the sites were randomly assigned to climate change simulation plots (hereafter, CCS plots) where we manipulated temperature and precipitation, while the other half of the sites were assigned to control plots that were

Table 1
Short term changes (period 2021–2040) in air temperature and precipitation predicted for the study site during the oak recruitment season (October–February). Predictions were performed with the HadGEM2-ES model considering the four climate change scenarios (RCP2.6, RCP4.5, RCP6.0 and RCP8.5) proposed in the latest IPCC report.

Climate change scenario	Air temperature (net change)	Precipitation (percent change)
RCP2.6	+1.7 °C	−18%
RCP4.5	+1.2 °C	−14%
RCP6.0	+1.3 °C	−11%
RCP8.5	+1.3 °C	−16%

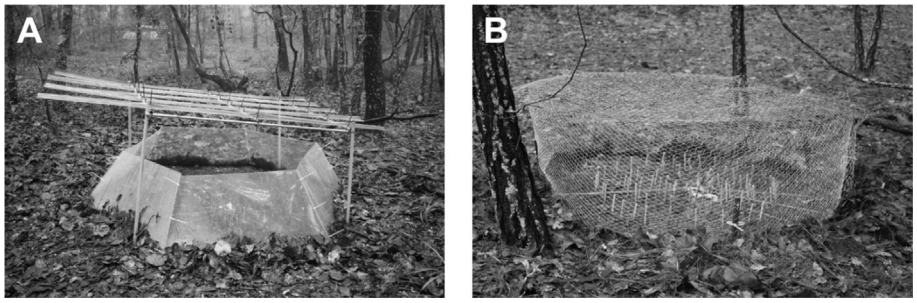

Fig. 1. Climate change simulation plots (CCS) consisting in open-top chambers covered with rainout shelters (A) and control plots fenced with wire mesh (B).

maintained under the current environmental conditions. To manipulate temperature in CCS plots we used hexagonal open-top chambers (Marion et al., 1997), which were built with sheets of transparent acrylic (3 mm thick, wavelength transmission 280–750 nm). The resulting open-top chambers were 0.50 m tall, 1.5 m wide at the open-top, and 2.08 m wide at the base (Fig. 1A). Previous studies indicate that this open-top chamber design can increase air temperature by 1–3 °C relative to the external environment, without affecting light transmission within the chambers (Dabros and Fyles, 2010; Aragón-Gastélum et al., 2014). Precipitation within CCS plots was reduced with rainout shelters (Yahdjian and Sala, 2002), which consisted in five U-shaped channels of transparent polycarbonate (1.5 mm thick, 10 cm wide, 3 m length) equidistantly arranged on metallic frames in order to cover 15% of the open-top of chambers (Fig. 1A). Before the experiment, we tested whether rainout shelters reduced precipitation within the ranges predicted for the study site (see above). These trials indicated that these shelters reduced precipitation by 10–20% at each rainfall event. Control plots, on the other hand, were fenced with wire mesh to prevent the access of granivorous and herbivorous vertebrates, such as mice, squirrels and birds (Fig. 1B).

Oak seedlings were developed in the field by sowing acorns within the former experimental plots. We only included two dominant oaks of the study site (*Q. crassifolia* and *Q. eduardii*) because the third species (*Q. jonesii*) did not produce acorns in 2015. To gather acorns of *Q. crassifolia* and *Q. eduardii*, we marked ten trees of each species with developing fruits on 09 August 2015 and removed leaf litter beneath their canopies. On 27 September 2015, after acorns were released from parental trees, we collected 100 mature fruits beneath the canopy of each of them. Because acorns quickly lose viability due to desiccation or infestation by insects, they were taken to the laboratory and the float test was used to separate viable and unviable acorns (Gribko and Jones, 1995). For this, we removed acorn cupules and placed the nuts in 20 L containers filled with water. After 2 h in water, we assumed that sunken acorns were viable and floating acorns were unviable (Gribko and Jones, 1995). This resulted in more than 600 viable acorns of each species, which were stored at 5 °C to prevent viability loss due to desiccation.

After acorn collection, we weekly visited the study site to assess the moment in which natural emergence of oak seedlings started in the field. This occurred on 14 October 2015 and, at this date, we randomly selected 200 viable acorns of each oak species and placed them in water during 48 h. We did this because acorns require to reach their maximum hydration capacity to germinate (Zavala-Chávez and García-Moya, 1996; Zavala-Chávez, 2008). These acorns were sowed on 17 October 2015. For this, we randomly assigned five controls and five CCS plots to each oak species and sowed 20 acorns within them. Acorns were sowed 2 cm depth to mimic the behavior of scatter-hoarding mice, which are their main secondary dispersers in North American oak forests (Smallwood et al., 2001). Sowing positions were marked with small wood stakes and the emergence of aerial shoots was monitored every seven days until 27 February 2016. At each monitoring date, we also recorded whether emerged seedlings were alive. Seedlings were assumed to have died when their stems and apical buds were completely withered (González-Salvatierra et al., 2013). We also recorded the dates of leaf unfolding on each seedling because, if climate change alters their phenology, leaf age would be required to control for effects of time in the analyses of foliar functional traits described below. To avoid pseudo-replication, leaf ages (i.e., number of days elapsed after leaf unfolding) were averaged across leaves of each oak seedling.

2.3. Climate and soil conditions

To determine the extent of the microclimatic changes induced with open-top chambers and rainout shelters, as compared to current climate, we randomly selected three plots of each treatment (controls and CCS plots) and installed automatized sensors to measure temperature and relative humidity of the air, soil temperature and precipitation. Temperature and relative humidity of the air were measured with dual dataloggers fixed on wood stakes 20 cm above ground (HOBO U23-Pro-V2, Onset Computer Corporation, USA), while soil temperature was measured with dataloggers buried 5 cm depth (HOBO Pendant-UA-002, Onset Computer Corporation, USA). In this way, temperature was measured in the environments in which acorns germinate (belowground) and oak seedlings develop (aboveground). These dataloggers were programed to measure climatic variables every hour during the entire experiment. Precipitation was measured with automatized pluviometers programed to record this variable at each rainfall event (HOBO S-RGB-M002, Onset Computer Corporation, USA). All these sensors were arranged at the center of each plot taking care of avoiding interference among them.

We also measured soil volumetric water content during the entire experiment. For this, we randomly selected five experimental plots of each climate treatment and marked them. In these plots, we performed six point-measures every week using a time-domain reflectometer (FieldScout TDR 300, Spectrum Technologies, USA) and these values were averaged per plot at each measurement date. This latter variable was measured at 3.8 cm depth, corresponding to the environment in which acorns were sowed and can influence seedling emergence, and 12.0 cm depth, corresponding to the environment from which the roots of emerged seedlings can uptake water and nutrients.

To assess whether the climate change induction alters soil nitrogen content, we measured the concentrations of nitrate (NO_3^-) and ammonium (NH_4^+) in controls and CCS plots. We focused on NO_3^- and NH_4^+ because these are the mineral forms of nitrogen that plants can use (Fitter, 1997). Concentrations of NO_3^- and NH_4^+ were measured on the five plots of each climate treatment that we selected to perform water content measures. Nevertheless, as measuring NO_3^- and NH_4^+ required to extract soil samples and this could damage oak seedlings, these measurements were only conducted at the beginning (before climate manipulation) and at the end (after climate manipulation) of the experiment. At both times, four topsoil cores (5 cm depth, 2 cm width) were collected within each experimental unit and they were pooled in a mixed sample. This sample was meshed (2 mm mesh) and its NO_3^- content was determined in the field with portable ion-

selective meters (LAQUATwin NO_3^- meter B-742, Horiba, Japan). This procedure was repeated three times for each soil sample and NO_3^- readings were averaged. The remaining fraction of each soil sample was placed in a hermetic plastic bag and stored in ice to reduce the metabolic activity of soil microorganism. After arriving to the laboratory (about 3 h after soil collection), these soil samples were spread on aluminum sheets and dried in an air-forced stove at 40 °C until their weight remained constant (about 48 h). Once samples were fully dry, we took 5 g of soil and NH_4^+ was extracted with 2 M potassium chloride solution. These extracts were treated with Berthelot's reagent and read in a UV–visible spectrophotometer (Genesys 10S, Thermo Fisher Scientific). This procedure was repeated three times for each soil sample and NH_4^+ values were averaged.

2.4. Oak seedling responses

At the end of the experiment, ungerminated acorns were recovered from the experimental plots and taken to the laboratory to determine their viability. For this, we made a small incision on the pericarp of acorns and incubated them in 1% triphenyltetrazolium chloride solution during 24 h in dark. If seed embryos are alive, the colorless tetrazolium salt reacts with the hydrogen released by active dehydrogenase enzymes and this generates formazan, a non-diffusible red dye (Bewley et al., 2013). As no recovered acorn was viable (i.e., the embryos were not red-stained), we assumed that no additional germination would occur after the experiment was finished. These data were used to compare the proportion of germinated acorns and surviving seedlings between controls and CCS plots. At this time, we also measured the length of the aerial shoots of all surviving seedlings (i.e., length from the ground to the apical bud) with a digital caliper. These data were used to estimate their net growth rates as the ratio between the length of the aerial shoot and the age of each seedling (i.e., the number of days elapsed after shoot emergence) and these values were compared between climatic treatments.

We later assessed a series of foliar functional responses on three surviving seedlings from each experimental plot to determine whether climate change can affect their performance. All these seedlings had at least three mature leaves without symptoms of wilting or insect damage, which were marked and numbered with small paper tags. In the field, we used a portable fluorometer (MINI-PAM II, Heinz Walz, Germany) to assess foliar temperature and photosynthetic photon flux density (*PPFD*) on each leaf, and estimated the effective quantum yield (ϕ_{PSII}) by measuring their chlorophyll fluorescence. Values of ϕ_{PSII} were computed as $(F'm - Ft) / F'm$, where F_t is the fluorescence emitted by leaves under steady-state illumination and F'_m is their maximum fluorescence when a saturating pulse of actinic light ($\approx$2500 μmol photon m^{-2} s^{-1}, in our case) is superimposed on the environmental light level (Maxwell and Johnson, 2000). These measurements were conducted between 10:00 and 12:00 h because, after midday, the elevated levels of light could mask the effects of other environmental factors on photosynthetic quantum yield (Franco and Lüttge, 2002). Values of leaf temperature, *PPFD* and ϕ_{PSII} were averaged across leaves belonging to the same seedling to avoid pseudo-replication.

Tagged leaves were later harvested, stored in hermetic plastic bags and placed in ice to reduce water loss. These leaves were weighed immediately after they arrived at the laboratory (about 3 h after harvesting) to determine their fresh biomass (*FB*). After that, leaves were dried in an air-forced stove at 60 °C until their dry biomass (*DB*) remained constant (about 24–36 h). Values of *FB* and *DB* were determined using an analytical balance with resolution of 0.1 mg (Cubis, Sartorius, Germany) and used to estimate the percent water content (*PWC*) of leaves as $(FB - DB) / FB$. These values were averaged across the leaves of each seedling and used as surrogate of their water status at the moment of harvesting (Gond et al., 1999). Further, to assess whether climate change can affect the carbon and nitrogen balance of recently emerged oak seedlings, dried leaves were ground in a ball mill (MM200, Retsch, Germany) and processed to determine the percent contents of carbon and nitrogen with an elemental analyzer (ECS 4010, Costech, USA). Because of their small size, leaves collected on each seedling were pooled in a single sample to reach the minimum biomass (0.8 g) required for these analyses.

2.5. Statistical analysis

We used repeated measures ANOVAs to compare all environmental variables between climate treatments. For these analyses, values of air and soil temperature, as well as values of relative humidity of the air, were averaged at each week of the experiment for each datalogger (n = 3 per climate treatment; repeated measures = 19 weeks). On the other hand, precipitation recorded by each pluviometer was accumulated for every week of the experiment (n = 3 per climate treatment) but, as ANOVAs does not admit groups with zero variance (Zar, 2010), we excluded those weeks with no rainfalls from this analysis (repeated measures = 11 weeks). Comparisons of soil water contents at 3.8 and 12.0 cm depth were conducted with the values measured every week of the experiment (n = 5 per climate treatment; repeated measures = 19 weeks), while soil concentrations of NO_3^- and NH_4^+ were compared by only including the two dates in which these variables were measured (n = 5 per climate treatment; repeated measures = 2 dates).

The proportion of germinated acorns and surviving seedlings were compared between climate treatments and oak species with two-way factorial ANOVA (2 climate treatments x 2 oak species = 4 treatments in total; n = 5 per treatment). The identity of oaks was included in these analyses because responses to climate change may vary between species. The same analyses were used to compare the age of seedlings, their net growth rates and leaf ages, *PWC*, foliar temperature, *PPFD*, ϕ_{PSII}, and foliar contents of nitrogen and carbon. The values of all these variables were averaged across seedlings from the same experimental unit to avoid pseudo-replication. All datasets that support the former analyses were deposited in Mendeley Data (https://doi.org/10.17632/bsvxh88ypz.1).

Table 2
Results of repeated measures ANOVA addressed to compare climatic variables and soil features between climate treatments. The table shows the F-values obtained for each factor (climate treatments and time) and their interaction. Significant effects on each response variable are indicated with an asterisk (critical α = 0.05).

Environmental variable	Climate treatment	Time (weeks)	Interaction climate treatment-time
Air temperature	$F_{(4,1)} = 853.759$*	$F_{(18,72)} = 5178.385$*	$F_{(18,72)} = 3.163$*
Precipitation	$F_{(1,4)} = 339.846$*	$F_{(8,32)} = 10846.486$*	$F_{(8,32)} = 31.435$*
Soil temperature	$F_{(1,4)} = 5310.901$*	$F_{(18,72)} = 1482.021$*	$F_{(18,72)} = 0.229$
Air relative humidity	$F_{(1,4)} = 1076.431$*	$F_{(18,72)} = 14174.897$*	$F_{(18,72)} = 109.919$*
Soil water content-3.8 cm depth	$F_{(1,8)} = 180.184$*	$F_{(18,144)} = 1722.143$*	$F_{(18,144)} = 32.258$*
Soil water content-12.0 cm depth	$F_{(1,8)} = 743.359$*	$F_{(18,144)} = 1522.408$*	$F_{(18,144)} = 35.600$*
Soil nitrate concentration	$F_{(1,8)} = 181.203$*	$F_{(1,8)} = 562.389$*	$F_{(1,8)} = 299.475$*
Soil ammonium concentration	$F_{(1,8)} = 405.474$*	$F_{(1,8)} = 1111.181$*	$F_{(1,8)} = 577.643$*

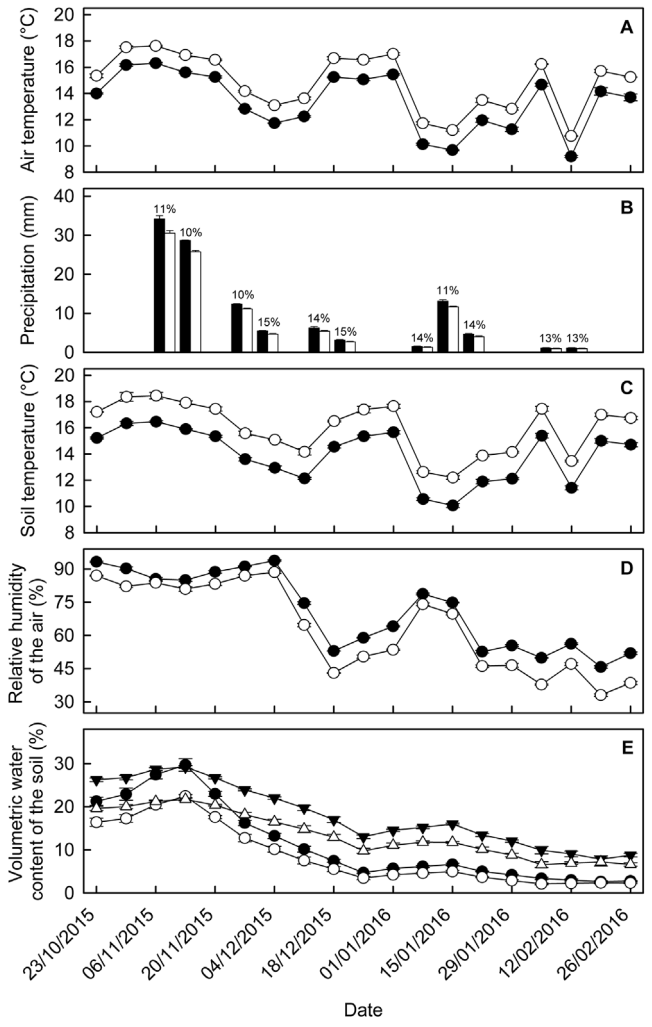

Fig. 2. Average values (± 95% C.I.) of air temperature (A), precipitation (B), soil temperature (C), relative humidity of the air (D), and soil water content (E) at each week of the experiment in controls (black symbols) and CCS plots (white symbols). Numbers above precipitation bars indicate the percent difference between climate treatments. Soil water content is reported at 3.8 cm (circles) and 12.0 cm depth (triangles).

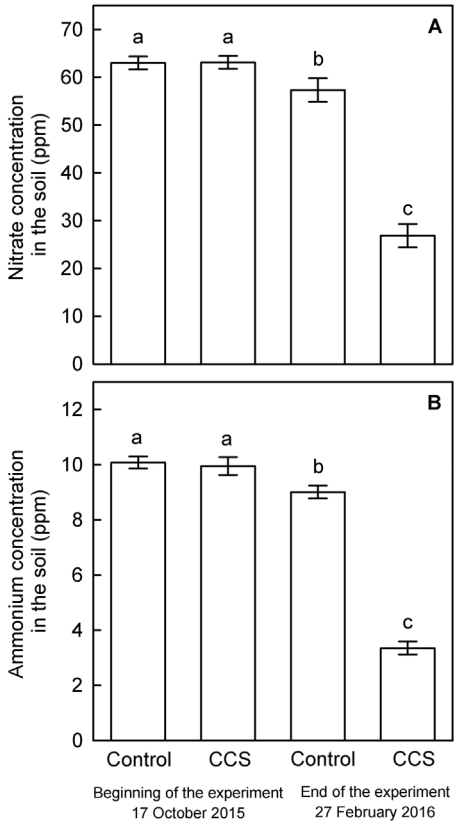

Fig. 3. Soil concentrations (average ± 95% C.I.) of nitrate (A) and ammonium (B) in controls and CCS plots at the beginning (17 October 2015) and at the end of the experiment (27 February 2016). Different letters above the bars indicate significant differences between treatments (Tukey tests critical $\alpha = 0.05$).

3. Results

3.1. Climate and soil conditions

Air temperature was higher in CCS plots than in controls across the entire experiment, while the converse occurred with precipitation (Table 2). Differences in air temperature between treatments increased from the beginning to the end of the experiment; CCS plots were about 1.3 °C warmer than controls until 18 December 2015, but this difference increased up to 1.5 °C after that date (Fig. 2A). Precipitation was 10–11% lower in CCS plots than in controls on those weeks where rainfalls surpassed 10 mm, while CCS plots received 14–15% less precipitation on those weeks where rainfalls were below that value (Fig. 1B).

Soil temperature, relative humidity of the air and soil water contents also differed between climate treatments (Table 2). Soil temperature within CCS plots was about 2.0 °C higher than in controls and this difference was sustained along the entire experiment (Fig. 2C). Air relative humidity was always lower in CCS plots than in controls, but these differences decreased in rainy weeks (Fig. 2D). Water contents at both, 3.8 cm and 12.0 cm depth, were lower in CCS plots that in controls and these differences increased when rainfalls decreased (January–February; Fig. 2E).

Soil concentrations of NO_3^- and NH_4^+ differed between climate treatments and sampling dates (Table 2). At the beginning of the experiment (i.e., before climate manipulation was applied), there were no differences in the concentrations of NO_3^- (Fig. 3A) or NH_4^+ (Fig. 3B) between plots assigned to controls and CCS. However, concentrations of both mineral nutrients were lower in CCS plots than in controls by the end of the experiment (Fig. 3).

3.2. Seedling responses

The proportion of emerged and surviving seedlings differed between climate treatments (emergence: $F_{(1,16)} = 18.086$, $p = 0.006$; survival: $F_{(1,16)} = 50.585$, $p < 0.001$) and oak species (emergence: $F_{(1,16)} = 60.409$, $p < 0.001$; survival: $F_{(1,16)} = 152.669$, $p < 0.001$). Interactive effects of these two factors (climate treatment x oak species) were also found on both response variables (emergence: $F_{(1,16)} = 7.763$, $p = 0.013$; survival: $F_{(1,16)} = 108.411$, $p < 0.001$). Emergence of *Q. crassifolia* seedlings was lower in CCS plots than in controls, while emergence of *Q. eduardii* seedlings did not differed between climate treatments (Fig. 4A). However, in both controls and CCS plots, the proportion of emerged seedlings was higher for *Q. eduardii* than for *Q. crassifolia* (Fig. 4A). The proportion of surviving seedlings in controls did not differ between oak species, but seedlings of *Q. eduardii* had higher survival than those of *Q. crassifolia* in CCS plots (Fig. 4B).

Age of surviving seedlings neither differed between climate treatments nor oak species, averaging 102 days across all experimental units (data not shown). Conversely, shoot growth rates differed between climate treatments ($F_{(1,16)} = 346.225$, $p < 0.001$) and oak species ($F_{(1,16)} = 9.181$, $p = 0.008$), but no interactive effects between these two factors were found ($F_{(1,16)} = 0.113$, $p = 0.741$). This analysis

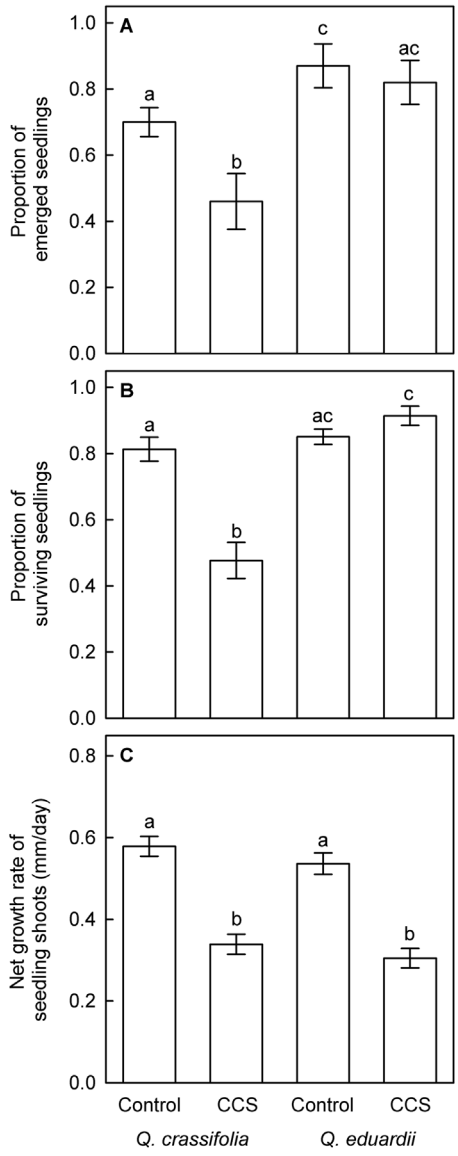

Fig. 4. Proportion (average ± 95% C.I.) of emerged seedlings (A) and surviving seedlings (B) of *Q. crassifolia* and *Q. eduardii* in controls and CCS plots. The figure also shows the average (± 95% C.I.) net growth rates of seedling shoots (C) in both climate treatments. Different letters above the bars indicate significant differences between treatments (Tukey tests critical $\alpha = 0.05$).

indicated that seedlings of both, *Q. eduardii* and *Q. crassifolia*, had higher shoot growth rates in controls than in CCS plots (Fig. 4C).

Age of seedling leaves differed between climate treatments ($F_{(1,16)} = 38.267$, $p < 0.001$) and oak species ($F_{(1,16)} = 8.152$, $p = 0.011$), and significant effects were also found for the interaction of these two factors ($F_{(1,16)} = 5.060$, $p = 0.039$). In this case, leaf unfolding occurred latter in *Q. crassifolia* seedlings than in seedlings from all the other treatments (Fig. 5A). For this reason, leaf age was included as continuous concomitant variable in the analyses comparing the other foliar functional traits (i.e., we used ANCOVA instead of ANOVA). Values of *PWC* were not affected by leaf age, but significant differences were found between climate treatments and oak species (Table 3). Leaves of both oak species had lower *PWC* in CCS plots than in controls and these differences were higher for *Q. crassifolia* than for *Q. eduardii* (Fig. 5B). Neither leaf age nor oak species affected foliar temperatures (Table 3), but both oak species had higher foliar temperatures CCS plots than in controls (Fig. 5C).

Values of *PPFD* neither differed between climate treatments nor oak species (data not shown), indicating that leaves of all seedlings were exposed to similar levels of light (578.606 μmol $m^{-2} s^{-1}$ ± 6.643 95% I.C.) during the chlorophyll fluorescence measurements. Values of ϕ_{PSII} were not affected by leaf age and they did not differ between oak species (Table 3). However, seedlings both of oak species displayed higher ϕ_{PSII} values in controls than CCS plots (Table 3; Fig. 5D).

No effects of leaf age were found on foliar contents of nitrogen and carbon, but values of these two variables differed between climate treatments and oak species (Table 3). Both oak species had higher contents of foliar nitrogen (Fig. 5E) and foliar carbon (Fig. 5F) in controls than in CCS plots, and these differences were larger in seedlings of *Q. crassifolia* than in seedlings of *Q. eduardii*.

4. Discussion

Our study indicates that increasing temperature and drought will impair the development of recently emerged oak seedlings in the field and, as far as we are aware, this is the first experimental study addressing these issues in temperate forests of North America. Indeed, as open-top chambers and rainout shelters properly simulated the conditions of temperature and rainfall expected in the short term (2021–2040), our experimental results suggest that these negative impacts of climate change would occur much sooner than predicted by other studies, which have used species distribution models to simulate the future distribution of tree species and proposed that climate change effects on these forests will be evident after the middle of this century (Gómez-Mendoza and Arriaga, 2007; Mclaughlin and Zavaleta, 2012). The induction of higher temperature and lower rainfall in the field also modified other environmental variables that can impair the development of oak seedlings, and it is important to note that the future shifts in the values of these variables are not directly predicted by climate change models. For instance, we observed an overall decrease of air relative humidity within CCS plots, which can be attributed to the direct inverse relationship between the values of this variable and air temperature (Hardwick-Jones et al., 2010). Further, higher soil temperatures resulting from warming could have increased soil water evaporation within CCS plots (Parlange et al., 1998) and this, together with the smaller water inputs due to rainout shelters, may be responsible for the lower soil water contents recorded in these experimental units. In this way, the climatic changes that will occur during the next decades in temperate forest due to the increased emission of greenhouse gases may reduce the recruitment rates of oaks in these ecosystems. Nevertheless, our results also indicated that the two oak species included in this study somewhat differed in their responses to climate change.

Emergence and survival of *Q. crassifolia* seedlings were lower in CCS plots than in controls, while these differences were not found for *Q. eduardii*. This suggests that the early life-cycle stages of *Q. crassifolia* would be more sensitive to climate change than those of *Q. eduardii*. Because acorn of most oak species quickly lose viability when external factors enhance seed water loss (Zavala-Chávez, 2008; Joët et al., 2013), the higher soil temperatures induced in CCS plots may have accelerated acorn desiccation and, consequently, this may have reduced the emergence of *Q. crassifolia* seedlings. Further, as oak seeds require elevated hydration levels to stimulate the development of their embryos (Zavala-Chávez and García-Moya, 1996), the lower soil moisture contents in CCS plots may have prevented that the acorns of *Q. crassifolia* reached the water contents they needed to germinate. Thus, the combined effects of higher temperature and reduced rainfall may be responsible of the lower proportion of emerged seedlings of this oak species in CCS plots.

The lower survival of recently emerged *Q. crassifolia* seedlings in CCS plots can also be attributed to the combination of increased warming and drought. Several authors have reported that, under the current climatic conditions, oak seedlings quickly wilt if they face thermal and/or water stress during their recruitment season, which

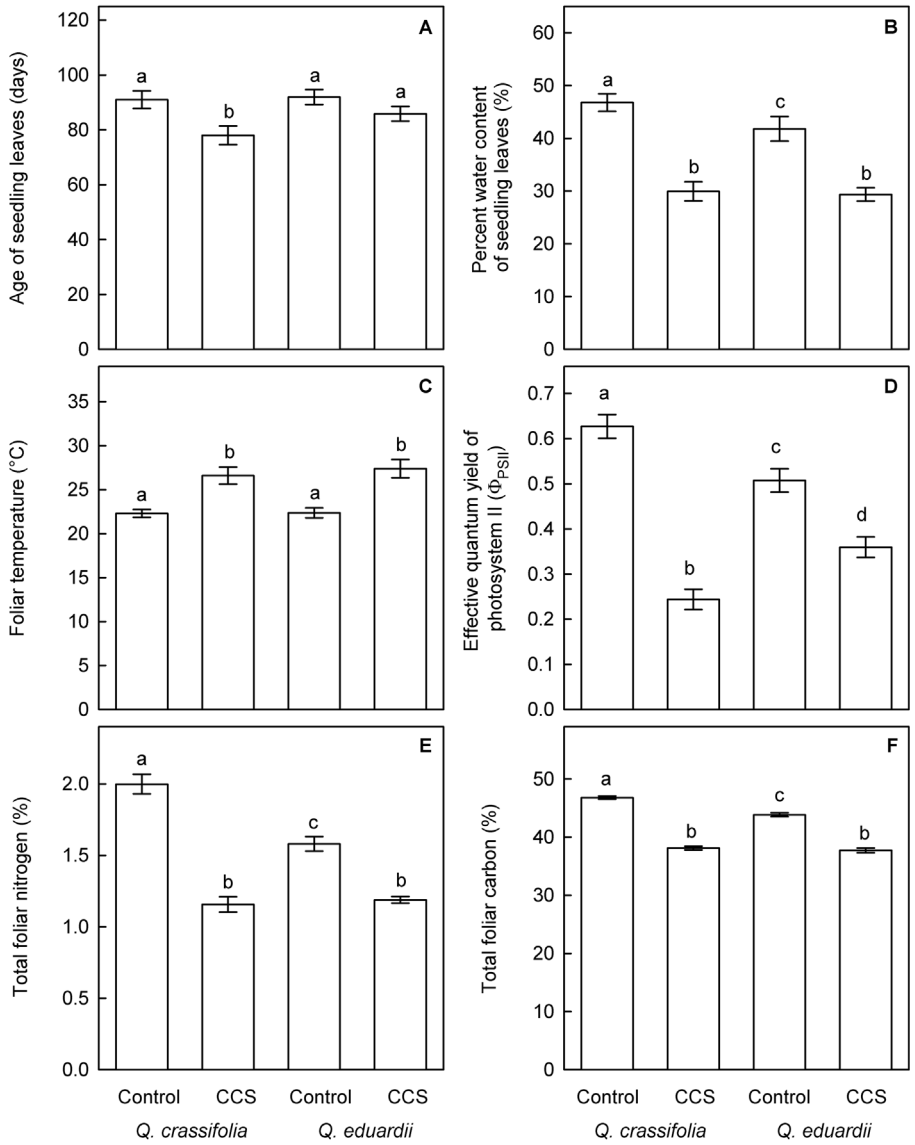

Fig. 5. Foliar functional traits (average ± 95% C.I.) of *Q. crassifolia* and *Q. eduardii* seedlings from controls and CCS plots, including age of leaves (A), leaf percent water content (B), foliar temperature (C), effective quantum yield of photosystem II (D), foliar content of nitrogen (E) and foliar content of carbon (F). Different letters above the bars indicate significant differences between treatments (Tukey tests critical α = 0.05).

drastically increases their mortality in the field (Rey-Benayas et al., 2005; González-Salvatierra et al., 2013; Montes-Hernández and López-Barrera, 2013; Badano et al., 2011, 2015). In our study site, climate change models predict that these two stress factors will intensify in the near future, and our experimental results indicate that such increased environmental harshness will reduce the survival of *Q. crassifolia* seedlings. However, neither emergence nor survival of *Q. eduardii* seedlings were reduced within CCS plots, as compared to controls, suggesting that this oak species may be able to deal with climate change.

These differences in seedling emergence and survival between *Q. crassifolia* and *Q. eduardii* could be attributed to differential capabilities they have for recruiting in contrasting habitats. In temperate oak forest of Mexico, *Q. crassifolia* is considered a late successional species whose seedlings mainly recruit in shaded understory habitats and, although they have an elevated competitive ability for soil resources in

Table 3
Results of ANCOVA comparing leaf traits and associated variables between climate treatments and oak species. The table shows the F-values obtained for each factor (climate treatments and time) and their interaction. Significant effects on each response variable are indicated with an asterisk (critical α = 0.05). In these analyses, leaf age was included as concomitant continuous variable.

Environmental variable	Leaf age	Climate treatment	Oak species	Interaction climate treatment-oak species
Leaf percent water content	$F_{(1,15)} = 1.501$	$F_{(1,15)} = 60.208$*	$F_{(1,15)} = 10.332$*	$F_{(1,15)} = 2.299$
Foliar temperature	$F_{(1,15)} = 0.285$	$F_{(1,15)} = 32.095$*	$F_{(1,15)} = 1.331$	$F_{(1,15)} = 1.061$
ϕ_{PSII}	$F_{(1,15)} = 1.397$	$F_{(1,15)} = 115.490$*	$F_{(1,15)} = 0.693$	$F_{(1,15)} = 60.504$*
Total foliar nitrogen	$F_{(1,15)} = 0.640$	$F_{(1,15)} = 142.040$*	$F_{(1,15)} = 40.424$*	$F_{(1,15)} = 48.803$*
Total foliar carbon	$F_{(1,15)} = 0.034$	$F_{(1,15)} = 501.276$*	$F_{(1,15)} = 60.225$*	$F_{(1,15)} = 37.492$*

comparison with seedlings of other tree species, they do not tolerate elevated temperatures or water shortage (Castillo-Lara et al., 2008). Instead, even when *Q. eduardii* is also a conspicuous species within these forest, this oak is considered a pioneer tree because it colonizes disturbed open areas, where temperature and drought are much higher than beneath forest canopies (Alfonso-Corrado et al., 2007). Thus, lack of effects of climate change on the emergence and survival of *Q. eduardii* seedlings may be due to adaptions they have for establishing in harsh environments.

Despite the higher emergence and survival of *Q. eduardii* seedling in CCS plots, as compared with those of *Q. crassifolia*, our results suggest that climate change will negatively affect the development of the early life-cycle stages in both species. For instance, aboveground growth of these seedlings was about twice higher in controls than in CCS plots. This contrasts with the results of experiments that have manipulated temperature in cold and temperate forests, which have indicated that warming can hasten aerial shoot development of tree seedlings (Danby and Hik, 2007; Xu et al., 2012; Piper et al., 2013). Nevertheless, experiments conducted in arid and semi-dry environments have reported that the occurrence of drought events during the plant recruitment season can strongly impair the growth rates of tree seedling (Valladares and Sánchez-Gómez, 2006; Mclaughlin and Zavaleta, 2012). Thus, the lower growth rates of oak seedlings in CCS plots could be due to this latter factor, as the environmental variables measured in these experimental units suggested that the combined effect of increased temperatures and reduced rainfalls generated strong soil water deficits.

Climate manipulation within CCS plots also induced other detrimental effects on oak seedlings, including reduced leaf water content (*PWC*), lower carbon accumulation in their leaves, and diminished photosynthetic quantum yield (ϕ_{PSII}). The lower *PWC* of seedlings in CCS plots can be linked with their higher foliar temperatures, which may have increased water loss through transpiration (Haldimann and Feller, 2004; Rodriguez-Dominguez et al., 2016). Further, the lower air relative humidity within CCS plots may have reduced the boundary vapor layer of leaves, also enhancing foliar transpiration (Martin et al., 1999; Will et al., 2013). Since leaf dehydration induces stomatal closure (Brodribb and Holbrook, 2003), these lower *PWC* could have led to reduced gas exchange and this may partially explain why seedlings from CCS plots had lower carbon contents than those from the controls. The reduced values of ϕ_{PSII} in these seedlings can also be related with their lower carbon contents, as thermal and/or water stress decrease ϕ_{PSII} in plants (Xu and Zhou, 2006; González-Salvatierra et al., 2013; Aragón-Gastélum et al., 2014; Badano et al., 2015) and causes energetic deficits for fixing carbon through the Calvin cycle (Baker and Oxborough, 2004; Xu and Zhou, 2006). Therefore, our results suggest that climate change can also impair the photosynthetic efficiency of oak seedlings.

The lower contents of NO_3^- and NH_4^+ in the soil CCS plots concur with the results of other experiments conducted in forest ecosystems, which showed that climate change may cause nitrogen deficits for plants (Rennenberg et al., 2009; Chen et al., 2011; Roa-Fuentes et al., 2015). Decreases in soil nitrogen content under climate change conditions have been mainly attributed to the detrimental effects of water deficits on the metabolic activity of nitrogen-fixing bacteria, rather than increasing temperatures (Borken and Matzner, 2009; Chen et al., 2011). This decreased availability of nitrogen in the soil may explain the lower foliar nitrogen contents of seedlings that grew within CCS plots. However, is also important to note that the lower soil moisture in these plots may have reduced the mobility of this mineral nutrient in the soil matrix and, consequently, impaired its uptake through the roots of seedlings. These effects of drought on the activity of nitrogen-fixing organism and/or the mobility of nitrogen in the soil matrix, may explain the lower growth rates of oak seedlings in CCS plots, as nitrogen is a key nutrient that regulates plant development (Rennenberg et al., 2009; He and Dijkstra, 2014).

Overall, this study indicates that climate change conditions that will occur across temperate forest of North America have the potential to reduce the recruitment of oak species. Under the current climate, elevated temperatures and drought events that occur during the growing season of oaks constitute important ecological barriers for their establishment in temperate forest (Zavala-Chávez and García-Moya, 1996; González-Salvatierra et al., 2013; Montes-Hernández and López-Barrera, 2013; Badano et al., 2009, 2011, 2015), and our results reveal that the strengthening of these stress factors will impair even more the development of recently emerged oak seedlings. This, in turn, may have strong detrimental effects on the natural regeneration processes that regulate the maintenance of these forests, and this can threat the provisioning of ecosystem services that support human well-being.

5. Conclusion

Our results indicate that the advance of climate can reduce the emergence, establishment, growth and physiological performance of oak seedlings in temperate forest. Nevertheless, the differences in seedling emergence and survival that we observed between the two oaks included in this study suggest that some species could be more susceptible to climate change (e.g., *Q. crassifolia*) than others (e.g., *Q. eduardii*). On this issue, extensive field experiments are still required to identify what tree species are vulnerable to climate change and what species will be able to cope with warming and drought. This would allow to focus conservation actions on those species highly sensitive to climate change, promoting their assisted migration towards zones climatically suitable for their development and hence ensure their prevalence. Conversely, those species with elevated resilience to climate change could be used for developing sustainable forestry and/or addressing restoration programs to recover forests in degraded areas.

Statement of authorship

CLPR and EIB conceptualized the study, designed the field experiment, gathered the data and performed the satistical analyses; JPRO, PDS, JF, DD and JAFC contributed with the measurement of seedling variables and foliar traits in the field and the laboratory; all authors contributed interpret the results and co-wrote the article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.actao.2018.08.006.

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